IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of

TOIVOLA et al

Serial No. 497,813

filed 25th May, 1983

for

"NOVEL TRI-PHENYL ALKANE AND ALKENE DERIVATIVES AND THEIR PREPARATION AND USE"

D E C L A R A T I O N

I, LAURI VEIKKO MATTI KANGAS, declare:

1. That I am a citizen of Finland of Riihipellontie 16 as. 16, 20300 Turku 30, Finland. I am a Master of Sciences at the University of Turku, and since 1st April 1977 I have been employed by Farmos Group Ltd., the assignees of the above identified application, in research into new drugs. I am co-inventor with Reijo Juhani Toivola, Arto Johannes Karjalainen, Kauko Oiva Antero Kurkela, Marja-Liisa Soderwall, Guillermo Luis Blanco and Hannu Kalervo Sundquist, of the subject matter of the above identified application, and I am the Lauri Veikko Matti Kangas who made the Declaration dated October 31st 1984 previously filed in support the this Application.

2. The following experiments have been carried out under my supervision to compare the antitumour effects of the known anti-estrogenic called clomifene with those of 4-chloro-1,2-diphenyl-1-[4-[2-(N,N-dimethylamino)ethoxy] phenyl]-1-butene, hereinafter identified as compound 7 and called compound 7 on page 28 et seq of the above identified application. Compound 7 is claimed in claim 9 of the said application. Clomifene and compound 7 were compared in their ability to suppress DMBA-induced breast cancer in rats. The experiments were carried out essentially as described in my last Declaration i.e. as follows, and the indicated results were obtained.

3. Breast cancer was induced into 45-50 days old female Sprague-Dawley rats by administering a single dose of 12 mg/kg of DMBA (dimethylbenzanthracene) in sesame oil. When using DMBA for the induction the tumours appeared first in the armpits, then on the neck, and furthermore in the sides of the animal. The induction took place in a special isolator (Metall und Plastic GmbH) from which the animals were transferred after about 3 weeks into the animal cages. The treatment began after the appearance of measurable tumours, approximately 5-7 weeks after the induction. The effect of compound 7 on the number of the tumours was studied and compared with that of clomifene. The compounds were dissolved in an aqueous solvent containing

Polyethylene glycol 3000	28.8 g/l
Tween 80	1.92 "
NaCl	8.65 "
Methyl-p-hydroxy benzoate	1.73 "
Propyl-p-hydroxy benzoate	0.19 "

Compound 7 was administered daily per os to the animals while the controls received the solvent. The treatment lasted for about 35 days. The tumours were detected by individual measurement. The width (w) and the length (l) of the tumours were measured and the volume of the tumour was calculated by the formula: $V = \pi w^2 l/12$. In addition to the size of the tumour, the weight of the animal and the number of tumours were also observed during this study. At the end of the study, when the animals were killed, samples were taken from some animals for histological and hormonal studies. The treatment dose of compound 7 and clomifene was 3 mg/kg. According to estrogen receptor binding studies and antitumour assays in vitro against MCF-7 cells, the doses used are equivalent. Both substances were given per os. The number of rats was 5 or 6 rats/group.

4. A very good method or evaluating the antitumour effect in the DMBA model cannot be found in the literature. The following criteria were used in this study:

1. The size of the tumour was evaluated by measurement, by comparing the length and the width with a measuring scale in front of the person carrying out the procedure.

2. The volume of the tumour was calculated by assuming the shape of the tumour to be half-oval and by using the formula $V = \pi w^2 l/12$. When length and width were the same magnitude, the tumour was assumed to have the shape of a hemisphere. The same formula applies in these cases.

3. The number of tumours was checked at each measurement. Each measurable tumour regardless of size was considered a tumour. If two tumours had grown together, the number of tumours was counted as if the tumours were still separate.

4. When evaluating the size of the tumours, the smallest tumour taken into account had the diameter of 0.3 cm (3 mm). If this size was measured only once, it was not considered a significant growth, because accuracy of measurement in the 0.2 - 0.3 cm range is not good and the effect of errors in the measurement is great on the size of the tumours.

5. The size changes of tumours during treatment was calculated and the tumour changes were classified as follows:

Class 1 = actively growing tumour with at least a four-fold increase in volume.

Class 2 = ± tumour, the size of which did not change during treatment or the size of which at the end of the treatment was <4-fold compared with the situation at the beginning, or the size of which had decreased during treatment but which even in the end held > 1/4 of its original volume.

Class 3 = regressing tumour, which had decreased to less than 1/4 of its original volume or had disappeared completely.

The numbers of tumours placed in the different categories can be statistically compared by the χ^2-test.

6. The numbers of tumours that had totally disappeared were recorded separately.

The t-test and the χ^2-test were used in the statistical analysis of the results.

5. The experiments, which are summarized in Tables 1 and 2 below, showed that the number of tumours increased less in the animals treated with compound 7 or clomifene than in the control animals. The effect of compound 7 is somewhat more potent than that of clomifene. The results calculated on the basis of tumour growth classification show that compound 7 possesses statistically a significant antitumour effect compared to the control.

Table 1

The antitumour effect of clomifene and compound 7 on DMBA-induced mammary carcinoma in the rat. The number of tumours that appeared and disappeared during five weeks' treatment.

Group	No. of animals	Number of tumours: Start	End	Dissappeared	Change/animal (mean ± sd)	p*
Control	6	17	26	0	1.5±1.0	
Compound 7, 3 mg/kg	5	13	13	1	0.0±0.7	<0.05
Clomifene, 3 mg/kg	6	15	19	1	0.7±0.8	NS

Compound 7 inhibited the appearance of new tumors in a statiscally significant manner, whereas the effect of clomifene was not statistically significant.

* difference from the control group calculated by the t-test.

Table 2

The number of tumours in various groups classified by the growth characteristics. Treatment time 5 weeks. Class 1 = growth > 4-fold, class 2 = size change negligible: class 3 = decrease to ≤ 1/4 of the original or disappearance.

Group	No. of animals	Classification 1	2	3	Comparison to control χ^2 (v = 1)	P
Control	6	14	10	0		
Compound 7 3 mg/kg	5	3	9	1	4.220	< 0.05
Clomifene 3 mg/kg	6	7	10	2	1.960	NS

Compound 7 changed the ratio of stable and regressing tumours to growing tumours in a statistically significant manner. Clomifene also changed this ratio, but the effect was less and was not significant.

It is concluded that compound 7 is a more effective antitumour compound in vivo than clomifene.

6. I conclude from these results that compound 7 is at least as effective against the aforesaid hormone-dependent tumours as the known drug clomifene.

7. The undersigned declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; further that these statements are made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment or both under Section 1001 of title 18 of the United States Code, and that such wilful false statements may jeopardise the validity of the Application or any Patent issuing thereon.

Lauri Kangas

LAURI VEIKKO MATTI KANGAS

Dated this 15 day of May 1985